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Short Communication

Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bitter gourd (*Momordica charantia* L.)

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Abstract

BACKGROUND: Bitter gourd (*Momordica charantia* L.) is an important vegetable crop in tropical countries, including China and India. A wide range of genetic diversity exists in India with respect to fruit morphology such as colour, size and exocarp. A diversity assessment conducted using different DNA marker systems amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers) will be helpful in the establishment of a broad-based description for improved germplasm curation and the identification of germplasm for genome mapping and breeding of bitter gourd.

RESULTS: Genetic relationships between 38 bitter gourd accessions were determined with the aid of 29 RAPD, 15 ISSR and six AFLP markers. Greater polymorphism was detected by AFLPs when compared with RAPD and ISSR analyses using the same germplasm array (RAPD 36.5%, ISSR 74.5% and AFLP 78.5% polymorphism). The average marker index (MI) values derived from the three different marker systems differed dramatically, indicating that they vary in their discriminatory power (AFLP > ISSR > RAPD). The AFLP markers used were only weakly correlated with ISSR ($r^2 = 0.007$) and RAPD ($r^2 = 0.04$) marker analyses, whereas a comparatively high correlation ($r^2 = 0.77$) was found between RAPD and ISSR marker systems.

CONCLUSION: The studies using RAPD and ISSR markers were not able to uniquely discriminate all the bitter gourd accessions examined, whereas AFLP analysis was discriminatory and allowed for a more complete dissection of unique differences among accessions of bitter gourd within and between collection sites.

Keywords: markers; germplasm curation; bitter melon; diversity

INTRODUCTION

Genetic diversity is important in plant breeding and is commonly measured by genetic distance or genetic similarity.[1] Morphological and biochemical markers tend to be restricted to relatively few traits, display a low degree of polymorphism, are often environmentally variable in their manifestation and can depend on the expression of several unlinked genes.[2] In contrast, molecular marker-based genetic diversity analysis has potential for assessing changes in genetic diversity over time and space.[3] A molecular marker is a nucleotide sequence corresponding to a particular physical location in the genome. It plays an essential role today in the study of variability and diversity.[4]

Bitter gourd (*Momordica charantia* L.) is an important vegetable crop in tropical countries, including China and India. A wide range of genetic diversity exists in India.[5] The fruit morphology varies greatly in colour, size and exocarp characteristics. Indian *M. charantia* var. *charantia* cultivars bear large fruits, whereas wild, free-living *M. charantia* var. *muricata* ecotypes develop small, round fruits.[6] In contrast, three distinct types occur in China: ecotypes bearing small (10–20 cm), extremely bitter fruits; ecotypes which develop comparatively long (30–60 cm), slightly bitter fruits; and ecotypes which produce moderately to strongly bitter, 9–12 cm long, triangular or cone-shaped fruits.[7] In Southeast Asia, small ecotypes enjoying worldwide cultivation are botanically designated as *M. charantia* var. *minima* Williams & Ng (fruits <5 cm in diameter) and *M. charantia* var. *maxima* Williams & Ng (fruits >5 cm

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in diameter).[8] Phenotypic and DNA marker-based (random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers) diversity analyses of Indian *M. charantia* cultigens of diverse geographic origins were conducted to determine their population structure and genetic relationships.[9–11] Genetic affinities among cultigens were defined by their geographic origin, suggesting that opportunities exist for broadening the existing Indian germplasm collection. The objective of the present study was to compare the efficiency of RAPD, ISSR and amplified fragment length polymorphism (AFLP) markers in detecting genetic diversity among 38 accessions of bitter gourd.

MATERIALS AND METHODS

Plant materials and DNA extraction

Thirty-eight morphologically and geographically distinct *M. charantia* L. accessions (Table 1) were collected from different Indian states and then grown in summer 2006 and maintained at the Research Farm of the Indian Agricultural Research Institute, New Delhi, India. All accessions examined herein were self-pollinated four times before evaluation. The total genomic DNA was extracted by the cytyltrimethylammonium bromide (CTAB) method[12] from young leaf tissue (i.e. terminal whorl) ground to a fine powder. DNA sample concentration was determined using a fluorometer employing a Hoechst dye (Hoefer, Inc., San Francisco, CA, USA), and the DNA samples were diluted to 25 ng μL^{-1} prior to polymerase chain reaction (PCR) amplification. The DNA marker analyses in bitter gourd were described in our previous studies.[9–11]

Data analysis

The NTSYS-pc Version 2.0 software package[13] was used to calculate Jaccard's similarity coefficients[14] between genotypes, and then dendrograms were constructed using an unweighted paired group method of arithmetic means (UPGMA) algorithm based on these similarity coefficients. Various primer and genetic population descriptors were calculated to define primer efficacy and to characterise differences among populations. The resolving power (R_p) of a primer was calculated as $R_p = \sum I_b$, where I_b describes relative band informativeness and takes the value $1-2(0.5-p)$, with p being the proportion of the 38 cultigens possessing the band.[15] The marker index (MI) is the product of the total number of loci per primer pair (n) and the arithmetic mean heterozygosity (H_{av}): $MI = nH_{av}$.[16] H_{av} is the product of the polymorphic loci (β) and the polymorphic heterozygosity (H_p) divided by the number of polymorphic loci (n_p): $H_{av} = \beta H_p/n_p$. In order to estimate the congruence among dendrograms (derived from RAPD, ISSR and AFLP marker information), cophenetic matrices for each marker and index type were computed and compared using the Mantel test.[17]

RESULTS AND DISCUSSION

The study reported herein estimated genetic diversity among cultigens acquired from five uniquely different agroecological growing areas of India. Since identical accessions were surveyed,[9–11] direct comparisons could be made among these studies.

A total of 208 amplicons (size varying between 200 and 3000 bp) were produced by examining 38 *M. charantia* accessions with 29 RAPD primers, of which 76 were polymorphic, with an average of 2.62 polymorphic fragments per primer[9] (Table 2) and an average polymorphism of 36.5%. Fifteen ISSR primers produced 125 amplicons (size varying between 150 and 2700 bp) in the accessions examined, of which 94 were polymorphic (74.7%), and the average number of bands observed per primer and the average number that were polymorphic were 8.33 and 6.30 respectively[10] (Table 2). Six AFLP primer pair combinations generated 519 reproducible fragments (size varying from 50 to 500 bp), and the number of polymorphic fragments for each primer was 67.30, representing 78.5% of the total fragments (86.5) per individual primer pair[11] (Table 2). This indicates that the bitter gourd accessions examined herein were genotypically different, since they were drawn from distinctly different ecosystems and are morphologically dissimilar.[5]

The relative efficiency of marker types for genetic analysis varies among crop species. Russell *et al.*[18]

Table 1. Bitter gourd (*Momordica charantia* L.) accessions from India used for RAPD-, ISSR- and AFLP-based diversity analysis

Accessions	Number of accessions	State/province of origin of landraces/cultivars
Jaynagar Sel-1, Gayeshpur Sel-1, Mohanpur Sel-215, Gayeshpur Sel-29, Nakhara, WBK-1, IC-2763, DBTG-1, DBTG-3, DBTG-5, DBTG-5-3, DBTG-9, DBTG-11, DBTG-13, DBTG-14, DBTG-101, DBTG-202 and DBTG-102	18	East India: Orissa, West Bengal, Assam, Jharkhand
Pusa Do Mausami-green, Pusa Do Mausami-white, Arka Harit, DBTG-2, DBTG-4, DBTG-5-1 and DBTG-103	7	North India: Rajasthan, Haryana, Gujarat, Uttar Pradesh
CO-1, Priya, Preethi, MDU-1, Dindigul Local, Mangalkudi Local, Arupokkatai Local, DBTG-6 and DBTG-12	9	South India: Tamil Nadu, Kerala
DBTG-7 and DBTG-201	2	Central India: Madhya Pradesh
DBTG-8 and DBTG-10	2	Northeast India: Meghalaya

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Table 2. Analyses of banding patterns generated by RAPD, ISSR and AFLP assays for 38 accessions of bitter gourd

Markers	Number of assay units	Number of bands	Number of bands per assay unit	Number of polymorphic bands	Number of polymorphic bands per assay unit[a]	R_p	MI
RAPDs	29	208	7.17	76	2.62 (36.5)	10.69	2.15
ISSRs	15	125	8.33	94	6.30 (74.7)	13.12	2.27
AFLPs	6	519	86.5	404	67.30 (78.5)	27.52	11.75

[a] Numbers in parentheses denote % of polymorphic assay units.

reported comparatively fewer polymorphic bands with AFLPs than with RAPDs (46.8 *vs* 66.3%) in a survey of 18 cultivated barley accessions. In bitter gourd, greater genetic diversity was detected by AFLPs when compared with previous RAPD and ISSR analyses using the same germplasm array (RAPD 36.5%[9] and ISSR 74.5%[10] polymorphism). While the average number of polymorphic bands per AFLP primer combination herein was 67.30, detection of polymorphisms per primer in RAPD (2.62)[9] and ISSR (6.30)[10] analyses was dramatically lower. Garcia-Mas *et al*.[19] observed similar differences in polymorphism when comparing AFLP (23.1%), RFLP (62%) and RAPD (18%) markers in melon (*Cucumis melo* L.). The increased level of discrimination witnessed herein was likely due in part to the number and comparatively high discriminatory power of AFLP markers employed, as well as the diverse nature of the germplasm examined.

The AFLP markers used were only weakly correlated with ISSR ($r^2 = 0.007$) and RAPD ($r^2 = 0.04$) marker analyses performed on the same accessions. However, a comparatively high correlation ($r^2 = 0.77$) was found between RAPD and ISSR marker systems, suggesting similarities in their discriminatory nature in the cultigens examined. The possible reasons may be that these two marker systems detect polymorphisms since both are dominant markers, the primers anneal arbitrarily and the fragments detected are of almost the same size. The low correlation between AFLP and RAPD or ISSR markers might be due to the nature of the genetic differences detected among these systems[20] and the number of primers used (i.e. a comparatively higher number in the case of RAPDs). The population heterozygosity can be characterised by marker genotype analysis, and thus quantitative approximations of marker utility are typically described by heterozygosity (H_p and H_{av}) estimators and marker index (MI) values (Table 2). The average MI values derived from the three different marker systems differed dramatically, indicating that they vary in their discriminatory power (AFLP > ISSR > RAPD). The comparatively high MI (11.75) derived from the AFLP system was mainly due to the higher number of polymorphic bands generated (404) in comparison with RAPDs (76) and ISSRs (94). Thus the AFLP marker system might have value for the genotyping of bitter gourd lines being considered for plant variety protection and/or registration.

UPGMA cluster analysis based on RAPD and ISSR markers grouped the 38 genotypes into two main cluster groups (Figs 1(a) and 1(b), groups I and II, nodes 1 and 2), with Jaccard's similarity coefficients ranging between 0.57 and 0.93 for the RAPD assay and between 0.48 and 0.91 for the ISSR assay. RAPD-based cluster analysis (Fig. 1(a)) grouped two genotypes (DBTG-101 and Mohanpur Sel-215) into one cluster group (group I, node 1), while cluster group II (36 genotypes, node 2) contained two subgroups. Subgroup 1 consisted of one accession (Jaynagar Sel-1, node 2) that was genetically distinct from the other 35 accessions (subgroup 2) examined. Similarly, ISSR-based cluster analysis (Fig. 1(b)) partitioned the accessions into two major groups containing the same accessions as those of RAPD-based analysis (i.e. groups I and II containing two and 36 accessions respectively). Group II could be further partitioned into two relatively distinct subgroups – subgroup 1 containing seven genotypes (DBTG-2, DBTG-3, Nakhara, DBTG-103, Dindigul Local, DBTG-102 and Arka Harit, nodes 2–5) and subgroup 2 containing 29 genotypes (node 6) – with Jaccard's similarity coefficients ranging between 0.68 and 0.78. Although there was a high correlation ($r^2 = 0.77$) between these two marker systems, the clustering of genotypes within groups was not that close when RAPD- and ISSR-derived dendrograms were compared. These differences may be attributed to marker sampling error and/or the level of polymorphism detected and to their coverage of the overall genome in obtaining reliable estimates of genetic relationships among accessions.[21]

UPGMA cluster analysis based on AFLP markers revealed two groups (Fig. 1(c), groups I and II). Group I contained four accessions (CO-1, DBTG-3, DBTG-5-1 and DBTG-5-3, node 1) that were distinctly different from the other accessions examined. Cluster group II was partitioned into five distinct subgroups (34 cultigens, subgroups 1–7, nodes 2–7). Cluster groupings in RAPD analysis[9] and ISSR analysis[10] of the same accessions were not concordant with geographic origin or gross morphological differences between accessions (i.e. varieties *charantia*/*maxima vs muricata*/*minima*). In contrast, AFLP markers classified the accessions more closely, except for a few based on geographic origin and gross morphological similarity. For instance, the mutant Pusa Do Mausami-white is derived from Pusa Do Mausami-green, and these cultigens are very similar morphologically except

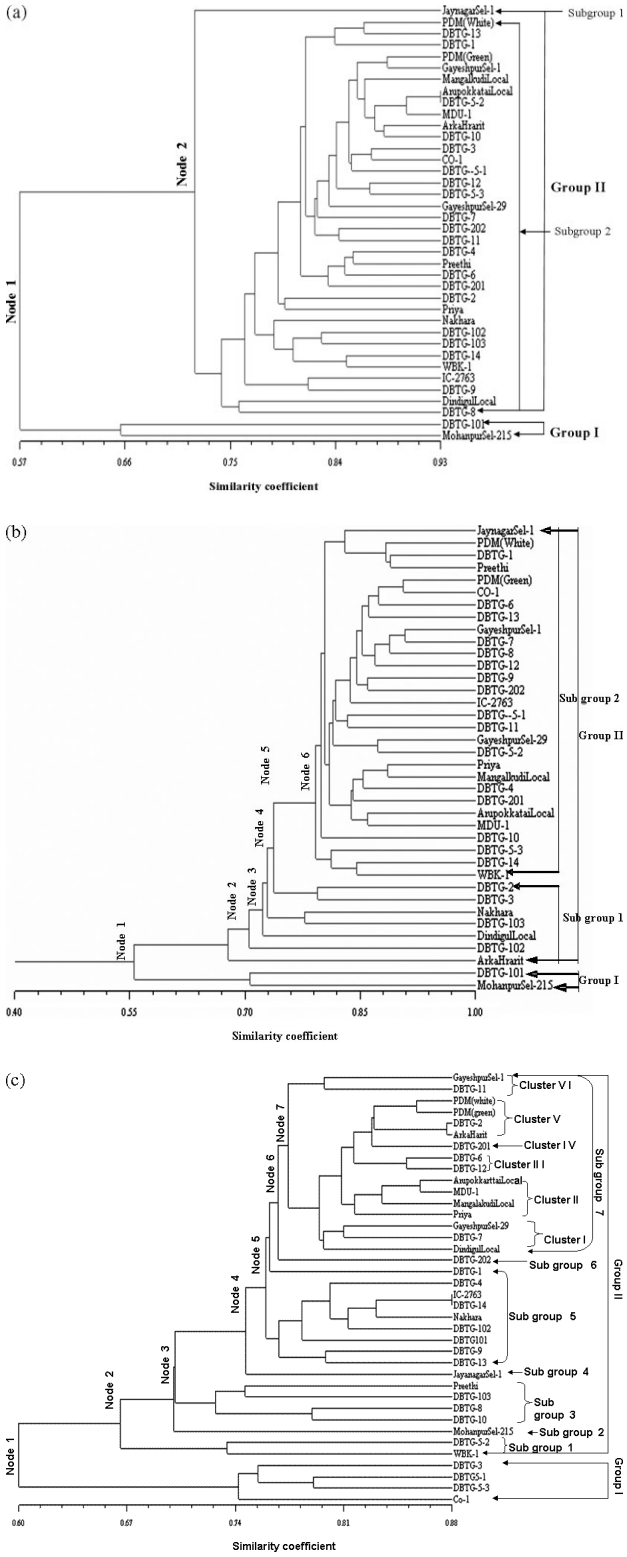

Figure 1. Genetic similarity relationships based on Jaccard's similarity coefficients[14] after cluster analysis of bitter gourd (*Momordica charantia* L.) accessions from India using (a) RAPD, (b) ISSR and (c) AFLP markers.

for a difference in exocarp colour, which is conditioned by a single locus. Similarly, eight accessions (DBTG-1, DBTG-9, DBTG-13, DBTG-14, DBTG-101, DBTG-102, Nakhara and IC-2763) out of nine appearing in subgroup 5 originated from East India, while four accessions (Priya, MDU-1, Mangalkudi Local and Arupokkatai Local) originating from South India were present in cluster II (subgroup 7). The studies using RAPD and ISSR markers were not able to uniquely discriminate all the bitter gourd accessions examined,[9,10] whereas AFLP analysis was discriminatory and allowed for a more complete dissection of unique differences among accessions within and between collection sites than in the previous studies.

The diversity assessment conducted herein provides additional markers for the establishment of a broad-based descriptive marker array (AFLP, ISSR and RAPD) for improved germplasm curation and the identification of germplasm for genome mapping and breeding of bitter gourd. The establishment of a discriminatory marker array is the first step to broader assessments of bitter gourd germplasm for genetic characterisation and the eventual development of a core collection of this species.[22,23] Based on genetic similarity estimates, a standard accession reference array for further analyses might include Pusa Do Mausami-green, Pusa Do Mausami-white, DBTG-2, Mohanpur Sel-215, DBTG-101, Nakhara, WBK-1 and Jaynagar Sel-1. As other collections of bitter gourd are made, genetic diversity can be assessed in relation to the standard accession reference array,[24] and additional germplasm groupings can be made based on morphology and DNA polymorphism such that a robust core collection can eventually be constructed.

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